From the standpoint of theory, the important observation has been made that the perrhenate ion does not inhibit, although its gross properties are otherwise very similar to those of the pertechnetate ion. Results of several studies will be presented soon in detail.

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RECEIVED MARCH 21, 1955

THE METABOLISM OF THIOCTIC ACID IN ALGAE

Sir:

Chromatographic separation of extracts from various photosynthetic organisms have shown several compounds that have the biological activity of thioctic acid (6T). The criterion for biological activity of these compounds was the response of propionate-inhibited *S. faecalis* grown on an acetate-free medium.[1] Chromatography in a mixture of butanol–ethanol–water gave the major biologically-active compounds at R_f's of 0.4, 0.7 and 0.9. The compounds at R_f = 0.4 and 0.7 were identified as thioctic acid sulfoxide and thioctic acid, respectively. The very lipid soluble compound at the front was not identified.

The synthesis of S^{35}-labeled 6T[2] has made possible a further investigation of thiotic acid metabolism in photosynthetic organisms.

0.5 g. of *Scenedesmus obliquus* suspended in 25 cc. 0.01 *M* phosphate buffer, *p*H 6.75, and containing 0.25 mg. of S^{35} 6T, was incubated in the dark aerobically. The distribution of the thioctic acid between algae and medium changed with time as follows: after 1 min., 16% in the cells; 10 min., 24%; 30 min., 42%; and 1 hour, 47%.

Such cells treated for at least one hour were extracted with ethanol and water and these extracts chromatographed in butanol saturated with 0.5 *N* ammonia. Five radioactive compounds were observed in the butanol–ammonia solvent at the R_f values: 0.98, 0.51, 0.33, 0.17, 0.1. The majority of activity was observed at R_f 0.98, 0.51 and 0.17. The latter two spots were identified as 6T and 6T sulfoxide. In a butanol–ethanol–water solvent system at least seven radioactive components could be observed. In long-term experiments the high R_f thioctic acid compound appeared to be a major component of the cells. To demonstrate that this compound was truly a metabolic product and not an artifact of the killing of the cells, extraction procedure, or chromatography, the S^{35} 6T was added to living algae which were then killed immediately and chromatographed. The results shown in Table I indicate clearly that this compound is a major metabolic constituent of the cell. An important observation is that it is not formed in any significant degree by cells under anaerobic conditions.

It has been shown by Bradley and Calvin[3] that thioctic acid must be metabolized aerobically by *Scenedesmus* cells before any stimulation of the Hill reaction could be observed. It was therefore of interest to see if there was any localization of this lipid compound in the cell in relation to the photochemical apparatus. S^{35} 6T-fed Chlorella (we have not yet succeeded in obtaining good plastid preparations from *Scenedesmus* but the total thioctic acid distribution in the two organisms is very similar) were ruptured by ultrasonication and chloroplast fragments isolated and washed. Both an extract of the plastids and the plastid-free cellular supernatant material were chromatographed. The results in Table II show clearly that, of the material in the chloroplast fragments, a large amount is aerobically-formed thioctic lipid.

TABLE I

Compound	S^{35} 6T	S^{35} 6T added and cells killed at once	5.25 hr. uptake in dark, aerobic
6T	91.4[a]	85	6
6T sulfoxide	5.4	5	14
Front	0.85	2.2	36
Origin	2.3	5.3	41

[a] Percentage distribution of total activity on chromatogram.

Table II

DISTRIBUTION OF COMPOUNDS IN CELL FRACTIONS

	Chloroplast fragments	Plastid-free supernatant
6T	7[a]	15
6T sulfoxide	5	8
Front	45	18

[a] Percentage distribution of total activity on chromatogram.

The very high R_f values and the behavior on alumina-column chromatography indicate that this conjugated thioctic acid is closely associated with the most lipid-soluble (hydrophobic) compounds in the cell and is readily converted back to thioctic acid by 4.0 *N* HCl, 1 hr., 120°. Further studies are underway to determine the structure of this thioctic-containing lipid and its possible relationship to photosynthesis.

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(2) P. Adams, Univ. Cal. Rad. Lab. Rep. U.C.R.L.-2949.

(3) D. F. Bradley and M. Calvin, *Arch. Biochem. Biophys.*, **53**, 99 (1954).

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(6) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

THE METABOLISM OF PROPIONATE BY RAT LIVER SLICES AND THE FORMATION OF ISOSUCCINIC ACID[1]

Sirs:

The pathways of propionate metabolism in mammalian tissues have not as yet been definitely established. According to one view, propionate is metabolized by conversion to acrylate and lactate,[2]

(1) Aided by a grant from the American Cancer Society.

(2) H. R. Mahler and F. M. Huennekens, *Biochim. Biophys. Acta*, **11**, 575 (1953).

and according to another view, by carboxylation to succinate.[3]

We have compared the metabolic patterns of propionate and lactate in rat liver slices with the aid of C^{14}-labeled compounds and a radiochromatographic technique.[4] In general the metabolic patterns of lactate resembled those of acetate. As was also observed by Daus, *et al.*,[5] propionate carbon appeared in all compounds labeled by acetate and, in addition, in β-hydroxyvalerate. However, the distribution of propionate carbon in these compounds differed greatly from that of lactate or acetate. Thus the incorporation of propionate carbon into fatty acids and ketone bodies was much lower, and that into glucose and especially in succinate was much higher than was the case with lactate.

The effects of malonate inhibition on the metabolism of propionate and lactate differed strikingly. As shown in Table I, malonate inhibited greatly

TABLE I

EFFECT OF MALONATE ON THE METABOLISM OF PROPIONATE AND LACTATE

200 mg. of rat liver slices incubated at 37° for 2 hours in 2 ml. of Krebs phosphate buffer, *p*H 7.6, in Warburg flasks with KOH in center wells. Gas phase, air. The flasks contained 10 μm. of propionate-1-C^{14} or 10 μm. of *dl*-lactate-1-C^{14}. Malonate concentration, 0.02*M*. The incorporation of C^{14} in the various compounds was determined as described in ref. 4.

		Per cent of added C^{14} in:					
Substrate	Malonate	CO_2	Succinate[b]	Beta hydroxyvalerate	Glucose	Alanine	Others[a]
Propionate-1-C^{14}	−	9.2	0.1	1.5	2.8	0.5	2.3
	+	1.6	0.9	1.4	0.1	0.1	1.0
Lactate-1-C^{14}[c]	−	22	0	0	0	13	0
	+	26	0	0	0	6	0

[a] Contains also isosuccinate. [b] Lactic and glutamic acids, glutamine, and small amounts of other compounds. [c] It should be noted that under other conditions, such as those used in ref. 4, lactate-1-C^{14} is incorporated into glucose, glutamate and other compounds.

the $C^{14}O_2$ yield from propionate-1-C^{14} but enhanced that from 1-C^{14}-lactate.[6] Also, in the case of the former, labeled succinate accumulated, but none whatsoever could be detected in experiments with lactate-1-C^{14}. Our results indicate that, in rat liver, propionate is not metabolized via acrylate and lactate. These results are, however, consistent with the carboxylation of propionate to succinate.

When the labeled spots on the chromatograms corresponding to succinate were eluted and rechromatographed with unlabeled succinate carrier, it was noted that although the radioactive area and the acid area as determined by indicator spray overlapped quite closely, the superimposition was not perfect. Upon closer study it was established that the "succinate" spot was composed of two acids; the major component was succinate and the smaller was another acid which moved with succinate in many common chromatographic systems. Under optimal conditions the mixture was resolved, and the minor component identified as isosuccinic (methylmalonic) acid. The best solvents for resolution of these two acids are described by Kalbe.[7]

C^{14}-labeled isosuccinate was isolated from a large scale experiment and its identity established by (a) co-chromatography with carrier isosuccinate in three different solvents; (b) crystallization to constant specific activity and (c) preparation of derivatives. In a series of three experiments, it was found that in the presence of malonate about twice as much succinate as isosuccinate is formed. Thus, it appears that rat liver contains enzyme systems that can add carbon dioxide to the β- and α-carbons of propionate, yielding succinate and isosuccinate, respectively.

Wolfe has shown (*Fed. Proc.*, 14-1, 306 (1955)) that in rabbit liver homogenate propionate is metabolized via succinate. The biosynthesis of isosuccinate from propionate and carbon dioxide by pig heart homogenates also has been reported by Flavin (*ibid.*, 14-1, 211 (1955)).

(3) H. A. Lardy and R. Peanasky, *Physiol. Res.*, **33**, 560 (1953).

(4) J. Katz and I. L. Chaikoff, *J. Biol. Chem.*, **206**, 887 (1954).

(5) L. Daus, M. Meinke and M. Calvin, *J. Biol. Chem.*, **196**, 77 (1952).

(6) J. Felts and M. J. Osborn in this laboratory have found that the addition of malonate increased greatly the incorporation of C^{14} of pyruvate-2-C^{14} into fatty acids by rat liver slices.

(7) H. Kalbe, *Z. physiol. Chem.*, **297**, 19 (1954).

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THE Si-F BOND MOMENT

Sir:

In a recent paper by Altshuller and Rosenblum[1] estimates of the dipole moments of the bonds H–Si, R–Si, and C–Si were based on a value of 2.3 *D* for the moment of the Si–F bond obtained from infrared intensity measurements on SiF_4.[2] While this value seems a not unreasonable one to choose for the static Si–F bond moment, the grounds for basing it on infrared intensity data are weak. Evidence is presented here that the infrared bond moment in SiF_4 is 3.3 *D* rather than 2.3 *D;* it is also emphasized that the infrared bond moment may be somewhat different from the static moment.

On the first point, it is to be recalled that the infrared intensity data yield two alternative values of μ_{SiF}, *viz.*, 2.3 and 3.3 *D*, respectively. A precisely similar situation exists in CF_4, SF_6 and in the in-plane vibrations of BF_3, the intensities of which have recently been measured.[3]

Table I shows the alternative values of μ and $\partial\mu/\partial r$ for these molecules. They have been collected into two sets, the first set containing the solutions having high values of μ and low and positive values of $\partial\mu/\partial r$. The strong resemblance between the individual solutions of set (1), and the irregularity of the solutions in set (2) favor the selection of set (1). In addition it is not possible to conceive of a simple mechanism whereby $\partial\mu/\partial r$ should be negative (with respect to μ, assumed to be positive, $^{+}X-F^{-}$) and of such magnitude as is found in set (2).

Further evidence exists in the case of BF_3 to support the higher bond moment. The intensity of the out-of-plane bending vibration yields a

(1) A. B. Altshuller and L. Rosenblum, THIS JOURNAL, **77**, 272 (1955).

(2) P. N. Schatz and D. F. Hornig, *J. Chem. Phys.*, **21**, 1516 (1953).

(3) D. C. McKean, unpublished work.